AD-A268 505

JMENTATION PAGE

Form Approved
OMB No. 0704-0188

2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
1993	Reprint

4. TITLE AND SUBTITLE

(see title on reprint)

5. FUNDING NUMBERS

PE: NWED QAXM

WU: 00162

6. AUTHOR(S)

Palazzolo, DL, and Kumar, KS

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Armed Forces Radiobiology Research Institute
8901 Wisconsin Ave.
Bethesda, MD 20889-5603

8. PERFORMING ORGANIZATION REPORT NUMBER

SR93-16

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Defense Nuclear Agency
6801 Telegraph Road
Alexandria, VA 22310-3398

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS	15. NUMBER OF PAGES 7
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		

Effects of S-2-(3-Methylaminopropylamino)ethyl Phosphorothioic Acid (WR-3689), Alone or Combined with Caffeine, On Catecholamine Content of Mouse Hypothalamus (43603)

D. L. PALAZZOLO[1] AND K. S. KUMAR
Department of Radiation Biochemistry, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20889-5603

Abstract. **S-2-(3-Methylaminopropylamino)ethylphosphorothioic acid (WR-3689) is a radioprotective agent that is behaviorally toxic at radioprotective doses. It was recently reported that the combination of WR-3689 and caffeine ameliorated behavioral toxicity (determined by locomotor activity in mice) compared with WR-3689 alone. Since catecholamines can modulate locomotor activity, we determined norepinephrine (NE) and dopamine (DA) content (using high-performance liquid chromatography) in the hypothalamus of mice after treatment with WR-3689, caffeine, and the combination of the two drugs. CD2F1 male mice were injected intraperitoneally with saline (control), WR-3689 (100 and 200 mg/kg), caffeine (20 and 40 mg/kg), or the combination of WR-3689 (200 mg/kg) and caffeine (40 mg/kg). Control values for NE and DA ranged between 200 and 220 pg/mg and 69 and 94 pg/mg of hypothalamic tissue, respectively. WR-3689 had no effect on the content of NE and DA. In contrast, NE increased to (mean ± SE) 324 ± 27 pg/mg and 377 ± 61 pg/mg ($P < 0.05$) 4 hr after injections of 20 and 40 mg/kg of caffeine, respectively. Similarly, DA increased to 142 ± 13 pg/mg ($P < 0.05$) 4 hr after injection of 40 mg/kg of caffeine. The combination of WR-3689 and caffeine had no effect on NE and DA contents when compared with control values. These results suggest that WR-3689 can affect catecholamine metabolism in the mouse hypothalamus, but the mode of action is not clear.** [P.S.E.B.M. 1993, Vol 203]

Phosphorothioates are effective radioprotectors (1). When these drugs are administered to laboratory rodents, the $LD_{50/30}$ of γ-irradiation is increased considerably. When given in doses that are efficacious against radiation, these drugs are also behaviorally toxic. One such compound, *S*-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721), is known to affect behavior in a number of species, including mice, rats, monkeys, and humans (2). When mice are treated with WR-2721, a significant decrement in locomotor activity is observed (3). WR-2721 impairs the ability of rats to maintain balance on an accelerod (4) and of monkeys to visually discriminate between tasks (5). In humans, common side effects of WR-2721 include nausea and vomiting, hypotension, hypocalcemia, and mild somnolence (6–8). Due to the nature of these toxic effects, there is reason to suspect that phosphorothioate-induced behavioral toxicity is derived from an imbalance in the central nervous system (CNS).

In clinical applications, the toxic side effects of phosphorothioates can be controlled. If these radioprotectants are to be used during space explorations, nuclear accidents, or when individuals must venture into radiologically hazardous environments, the toxic side effects of these drugs, particularly performance decrement, cannot be tolerated. If the side effects of phosphorothioates are eliminated without compromising radioprotection, these compounds could be used effec-

[1] To whom requests for reprints should be addressed at Radiation Biochemistry Department, Armed Forces Radiobiology Research Institute, 8901 Wisconsin Avenue Bethesda, MD 20889-5603.

Received October 26, 1992. [P.S.E.B.M. 1993, Vol 203]
Accepted March 4, 1993.

0037-9727/93/2033-0304$3.00/0

tively against the damaging effects of radiation in these scenarios (9).

Several analogs of WR-2721 have been synthesized and tested for radioprotection (10). Among these analogs, *S*-2-(3-methylaminopropylamino)ethylphosphorothioic acid (WR-3689), a methylated derivative of WR-2721, proved to be less toxic and clearly superior in some respects than WR-2721 (11). Studies reported in this paper were done using WR-3689.

In a recent study (12), the methylxanthine caffeine was administered to mice in combination with WR-3689 in an attempt to reduce its behavioral toxicity. The study indicated that the combination of WR-3689 and caffeine abolishes the decrement in locomotor activity normally observed in mice when WR-3689 is given alone. In addition, caffeine does not appear to alter the radioprotective efficacy of WR-3689 (13). Although the locomotor activity test is a better indicator of the tendency to perform, rather than the capacity to perform, it is still possible that the locomotor decrement produced by WR-3689 is a result of adverse alterations in the metabolism of neurotransmitters in the CNS. The catecholamines, in particular norepinephrine (NE) and dopamine (DA), are important neurotransmitters involved in the modulation of locomotor activity (14). Investigating the effects of phosphorothioates on catecholamines in the brain may provide a neurochemical basis for the phosphorothioate-induced decrement in locomotor activity.

The methylxanthines are potent stimulators of the CNS (15) and are known to affect catecholaminergic activity, but the mechanisms behind these effects are controversial and complicated. In the past, investigators have reported no changes (16–21), increases (17, 22, 23), and decreases (16) in absolute catecholamine levels in the brain after treatment with caffeine. Catecholamine turnover studies reveal similar ambiguities. There are reports indicating no changes (21), increases (17, 24–26), and decreases (17) in turnover in the brain.

The aim of the present study was to determine the effects of phosphorothioates and methylxanthines on the catecholaminergic system within the CNS. The content of NE and DA was assayed in mouse hypothalamus after treatment with WR-3689, caffeine, and the combination of the two drugs. The results of this study will help elucidate mechanisms involved in WR-3689- and/or caffeine-induced alterations of catecholamine metabolism as well as mechanisms involved in WR-3689-induced behavioral toxicity.

Materials and Methods

Animals. Male CD2F1 mice, 8–10 weeks old, were purchased from Charles River Laboratories (Boston, MA) and were housed five per cage in an air-conditioned (21 ± 1°C and 50 ± 10% relative humidity), light-controlled (lights on from 0600 to 1800 hr) American Association for Accreditation of Laboratory Animal Care-accredited facility. The mice were provided with food and water *ad libitum* and were maintained for at least 2 weeks before use in the experiments.

Drugs. WR-3689 was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) and anhydrous caffeine was obtained from Sigma Chemical Co. (St. Louis, MO).

Experimental Procedure. On the eve of experimentation, six groups of mice (five mice/group) were transferred to a nearby room to acclimatize them to new surroundings. At 0830 hr the following morning, intraperitoneal injections of neutralized saline (control; Group 1), WR-3689 (100 and 200 mg/kg; Groups 2 and 3), caffeine (20 and 40 mg/kg; Groups 4 and 5), and the combination of WR-3689 (200 mg/kg) and caffeine (40 mg/kg) (Group 6) were administered. One mouse from each group was sacrificed by quick cervical dislocation at the time of injection and the other four mice from each group were sacrificed at 1, 2, 4, and 8 hr after injection. Immediately after sacrifice, the hypothalamus was removed, weighed, and placed in 200 μl of 0.05 *M* $HClO_4$. The tissue was then sonicated (30 sec) and centrifuged in an Eppendorf microcentrifuge (3 min). The supernatant was stored at −80°C until the time of catecholamine analysis. The experiment was repeated eight times ($n = 8$ for each time point within each treatment group).

Analysis. Catecholamine concentrations were determined using high-performance liquid chromatography with electrochemical detection (Bioanalytical Systems, West Lafayette, IN) (27, 28). The high-performance liquid chromatography system incorporated an LC-4B electrochemical detector; a 10-cm, phase II, 3-μm octadecasilane reversed-phase column; and a glassy carbon working electrode. The mobile phase (pH 3.1) was made with degassed pyrogen-free water filtered through a Milli-Q purification system (Milipore Co., Bedford, MA) and included monochloroacetic acid (14.15 g/liter), sodium hydroxide (4.675 g/liter), disodium EDTA (150 mg/liter), octanesulfonic acid (300 mg/liter) as the ion-pairing agent, and 3.5% acetonitrile. The mobile phase was pumped through the system by a Beckman (Fullerton, CA) 112 solvent delivery module at a flow rate of 1.7 ml/min. The sensitivity of the detector was 2 nA full scale. The potential of the working electrode was 0.80 V with respect to a Ag/AgCl reference electrode. Standard and sample injections were made by a Rheodyne (Cotati, CA) 7125 syringe-loading injection valve. Standards for the catecholamine assays contained 600 pg of NE, DA, and isoproterenol per 50 μl of 0.05 *M* $HClO_4$. Isoproterenol served as the internal standard. Samples for the catecholamine assay contained 20 μl of homogenate and 30 μl of 0.05 *M* $HClO_4$ containing 600 pg of isoproter-

enol. Injection volume was 25 μl for both standards and samples.

Statistics. Data were collected with the use of a Shimadzu CR-4A Chromatopac (Columbia, MD). Mean values (±SE) are expressed as pg/mg wet wt of hypothalamic tissue. Comparisons between treatment groups at each time point were made using Student's *t* test, where $P < 0.05$ indicates statistical significance.

Results

Effects of WR-3689 and Caffeine Administered Individually on Hypothalamic Catecholamine Content. The effects of WR-3689 on the hypothalamic content of NE and DA are shown in Figure 1. Control values of NE and DA ranged between 200 and 220 pg/mg and 69 and 94 pg/mg of hypothalamic tissue, respectively. Administration of WR-3689 (100 and 200 mg/kg) had no effect on the content of NE or DA (Fig. 1). Figure 2 shows the effects of caffeine on the hypothalamic contents of NE and DA. NE content (Fig. 2) significantly increased ($P < 0.05$) above control values at 2 and 4 hr after administration of 20 mg/kg of caffeine and at all time points after administration of 40 mg/kg caffeine. The highest NE concentrations occurred 4 hr after caffeine treatment and were 324 ± 27 and 377 ± 61 pg/mg after the 20 mg/kg and 40 mg/kg injections, respectively. Compared with control values, DA (Fig. 2) was unaffected after treatment with 20 mg/kg of caffeine, but was significantly increased ($P < 0.05$) at 1 and 2 hr after administration of 40 mg/kg of caffeine. The highest DA concentration, 142 ± 14 pg/mg, occurred 2 hr after the 40-mg/kg injection of caffeine.

Effects of WR-3689 and Caffeine Administered Simultaneously on Hypothalamic Catecholamine Content. In Figure 3, the contents of NE and DA in the hypothalamus of mice treated with the combination of WR-3689 and caffeine are compared with the individual treatments of WR-3689 (200 mg/kg) and caffeine (40 mg/kg) alone. Catecholamine levels in the combined treatment group (200 mg/kg of WR-3689 and 40 mg/kg of caffeine) were not significantly different from the levels in the control group (data not shown), indicating that WR-3689 attenuates the caffeine-induced increase in catecholamines in mouse hypothalamus. NE and DA contents in the caffeine-treated group were significantly higher ($P < 0.05$) than in the WR-3689 treatment group at 2, 4, and 8 hr after

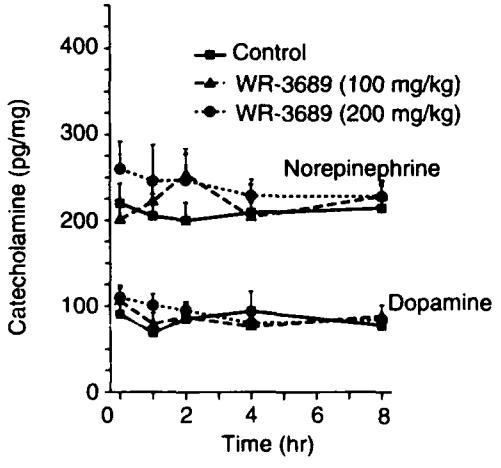

Figure 1. Effects of WR-3689 (100 and 200 mg/kg) on NE and DA content in the mouse hypothalamus. Control values are represented by the solid lines while 100-mg/kg and 200-mg/kg WR-3689-treated mice are represented by dotted and dashed lines, respectively.

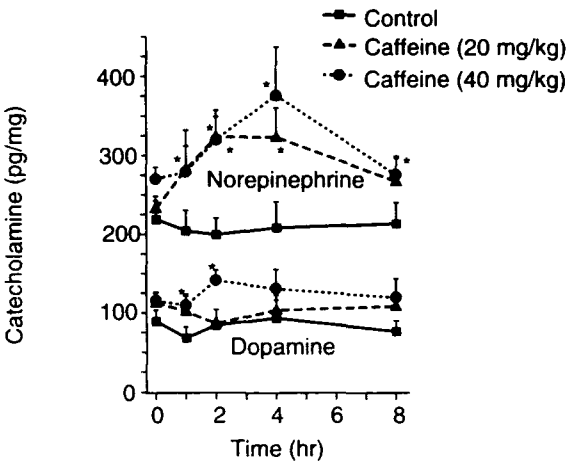

Figure 2. Effects of caffeine (20 and 40 mg/kg) on NE and DA content in the mouse hypothalamus. Control values are represented by the solid lines while 20-mg/kg and 40-mg/kg caffeine-treated mice are represented by dotted and dashed lines, respectively. *Significance ($P < 0.05$) between the control and caffeine treatment groups.

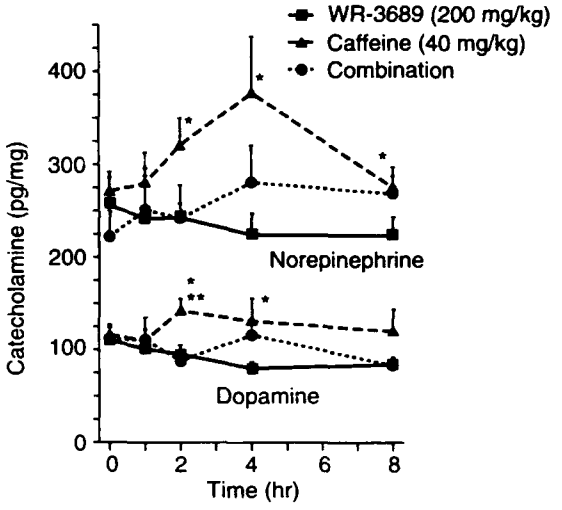

Figure 3. Effects of WR-3689 (200 mg/kg), caffeine (40 mg/kg), and the combination of the two agents at doses of 200 mg/kg and 40 mg/kg, respectively, on NE and DA content in the mouse hypothalamus. The WR-3689 treatment is represented by the solid line, the caffeine treatment is represented by the dotted line, and the combined treatment is represented by the dashed line. *Significance ($P < 0.05$) between the caffeine and WR-3689 treatment groups. **Significance ($P < 0.05$) between the caffeine and combined treatment group.

drug administration (Fig. 3). DA content in the caffeine-treated group was also significantly higher ($P < 0.05$) than in the combined treatment group 2 hr after drug administration. Although NE and DA contents in the combined treatment group were higher than the WR-3689-treated group and lower than the caffeine-treated group at 4 hr after injections, these differences were not statistically significant.

Discussion

The results of this study indicate that WR-3689 has no apparent effect on the hypothalamic content of NE and DA. This is consistent with prevailing thought that WR compounds do not readily cross the blood-brain barrier (BBB) (29, 30). Biodistribution studies indicate that only 0.1–0.2% of the total ^{35}S-radiolabeled WR-2721 (29) and WR-3689 (30) is accumulated in the brain 15–30 min after systemic injections. Since there were no differences in catecholamines between the controls and WR-3689-treated mice, it would seem that the small amount of WR-3689 taken up by the brain may not be sufficient to cause derangements in neuroendocrine processes. Alternatively, it is possible that WR-3689 could act centrally to affect synthesis and release of catecholamines in such a way that steady state levels of catecholamines remain unaltered. Dobric *et al.* (31) showed that WR-2721 (300 mg/kg, ip) antagonizes the effects of 9-gray γ-irradiation on the brain content of NE, DA, and serotonin. Deanovic *et al.* (32) reported a 40–60% decrease in the release of acetylcholine from the somatosensory cortex 15 min after systemic administration of WR-2721 (200 mg/kg). In addition, we found that simultaneous administration of WR-3689 and caffeine was effective in attenuating the increase in hypothalamic NE and DA content produced by administration of caffeine alone. These studies suggest that WR-3689 can alter neurotransmitter metabolism in the brain, but the mode of action remains to be established.

One explanation for phosphorothioate-induced alterations in catecholamine metabolism is that these agents could produce a general hypoxia (33, 34). If phosphorothioates indeed reduce oxygen tension, the resultant hypoxia could ultimately carry over into the brain. Once the brain becomes hypoxic, neurotransmitter metabolism can be altered (35–37). Another possibility accounting for the phosphorothioate-induced alterations in catecholamine metabolism is the inhibition of dopamine β-hydroxylase (38, 39) by sulfhydryl compounds such as glutathione and cysteamine. In a recent study investigating catecholamine metabolism in the mouse adrenal, we found an accumulation in the content of DA and a depletion in the content of NE and epinephrine 2–4 hr after administration of WR-3689 (40). These results indicate that WR-3689 inhibits dopamine β-hydroxylase. Vujnov *et al.* (41) reported similar decreases in NE in the adrenals and sera of rats 3 hr after WR-2721 administration. Interestingly, once the phosphate group is cleaved from the phosphorothioate, the aminothiol compound resembles cysteamine. In fact, it has been proposed that a small fraction of the administered phosphorothioate can be metabolized to cysteamine by various polyamine, diamine, or monoamine oxidases (42). Unlike phosphorothioates, cysteamine has no problem crossing the BBB (39) and is known to have a number of neuroendocrine effects (43, 44).

The increase in the hypothalamic content of NE and DA by caffeine was dose dependent. Govani *et al.* (22) found similar dose-dependent increases in the content of DA in the rat striatum and nucleus accumbens after administration of 10, 20, 40, and 100 mg/kg of caffeine. However, in the nucleus accumbens, the increase in DA content after injection of 100 mg/kg of caffeine was slightly less than the increase produced by 40 mg/kg, indicating a biphasic effect. Minana and Grisolia (23) administered caffeine to rats by way of the drinking water. At the end of their experiment, NE content in the hypothalamus and in the striatum was more than 300% above control levels, while the DA content was more than 100% above control levels. Corrodi *et al.* (17) found that 50 mg/kg of caffeine administered intraperitoneally slightly increased NE and DA stores in the whole brain of rats. In contrast, these authors reported a slight decrease in the content of NE and DA when 100 mg/kg of caffeine were injected. These results again indicate a biphasic nature of caffeine with the lower doses of caffeine increasing and the higher doses decreasing catecholamine content in the brain. In addition, a relationship between biphasic catecholamine levels and locomotor activity was established by Waldeck (18), who showed that lower doses of caffeine produce dose-dependent increases in locomotor activity, but the higher doses produce no effect or, in some instances, produce hypomotility.

In contrast, a number of studies investigating the effects of caffeine on the content of brain catecholamines report decreases (17) or no changes (16–21). Reasons for these discrepancies include the biphasic nature of caffeine, the time of tissue sampling after caffeine administration, and the use of whole brain preparations rather than discrete brain areas. In some studies, female rats were used without considering the stages of the estrous cycle. This is an important consideration since changes in the reproductive status of female rats are known to affect catecholamine activity (45).

Caffeine can alter catecholamine metabolism in the CNS by at least three modes of action (15). The first is associated with the translocation of calcium. Calcium is an essential ingredient of the secretion-coupling mechanism for the release of catecholamines

as well as other neurotransmitters and neuromodulators (46–48). Caffeine increases mobilization of calcium into the cell, resulting in increased catecholamine release. The second mode of action involves the accumulation of cAMP. The accumulation of cAMP is mediated by caffeine's ability to inhibit cyclic nucleotide phosphodiesterase. In turn, cAMP could increase catecholamine metabolism by stimulating tyrosine hydroxylase (49), the rate-limiting enzyme in catecholamine synthesis. The third mode of action involves blocking receptors for adenosine. Catecholamine release in the CNS can be mediated by specific adenosine receptors (50). Blocking adenosine receptors with caffeine leads to reduced catecholamine release. All three of these modes of action indicate that caffeine stimulates catecholamine metabolism, and this stimulation is in agreement with the results of the present study.

The attenuation of the caffeine-induced catecholamine content when caffeine was administered simultaneously with WR-3689 is intriguing, particularly when WR-3689 *per se* had no effect on the catecholamine levels. It is possible that caffeine alters the BBB via changes in calcium metabolism, permitting WR-3689 to enter the brain and thereby attenuating catecholamine levels. Another possibility is that WR-3689 enters the brain where the BBB is weak or absent. Two such areas are the organum vasculosum of the lamina terminalis, a circumventricular organ located at the tip of the third ventricle, and the area postrema, located immediately rostral to the obex on each side of the fourth ventricle (51). Furthermore, it is known that these two areas of the brain are involved in the regulation of body temperature (52) and in the control of the emetic response (53). It is also known that phosphorothioates alter body temperature (54) and trigger emesis (8).

If WR-3689 enters the brain, it can then interact with caffeine to attenuate catecholamine levels in a number of ways. (i) Phosphorothioates are known to cause a decrease in cytosolic calcium intrusion (55); thus, WR-3689 could reverse caffeine-induced intracellular mobilization of calcium that in turn affects catecholamine release. (ii) Tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis, is dependent upon molecular oxygen. Caffeine can stimulate tyrosine hydroxylase activity, presumably through the action of cAMP (49). If WR-3689 causes hypoxia (33, 34), it could limit the availability of oxygen for this rate-limiting step and hence catecholamine synthesis. (iii) Since phosphorothioates inhibit cAMP formation (56), it is possible that WR-3689 antagonizes the caffeine-induced accumulation of cAMP and, consequently, catecholamine levels. (iv) Caffeine can also stimulate dopamine β-hydroxylase in the hypothalamus (23). This stimulation could then offset the inhibiting effect sulfhydryls have on dopamine β-hydroxylase. (v) Caffeine is known to stimulate alkaline phosphatase (57, 58) that in turn can accelerate the formation of sulfhydryl from phosphorothioates. Although alkaline phosphatase is not detectable in brain, formation of the sulfhydryl from WR-3689 in peripheral blood could facilitate its entry into the brain. These observations could explain why WR-3689, when administered in combination with caffeine, attenuates the increase in hypothalamic catecholamines produced when caffeine is given alone.

The results of this study indicate that WR-3689 attenuates the caffeine-induced increase in hypothalamic concentrations of NE and DA, suggesting that WR-3689 affects catecholamine metabolism in the CNS. WR-3689, *per se*, has no effect on the hypothalamic stores of NE and DA, whereas caffeine alone increases the hypothalamic content of NE and DA in a dose-dependent manner. From these results, it is tempting to speculate that WR-3689-induced alterations in catecholamine metabolism are at least partially responsible for the WR-3689-induced behavioral toxicity.

This work was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under Work Unit 00162. Research was conducted according to the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Research Council. We are greatful to W. A. McLean for technical assistance and to Modeste Greenville for editorial assistance.

1. Giambarresi L, Jacobs AJ. Radioprotectants. In: Conklin JJ, Walker RI, Eds. Military Radiobiology. New York: Academic Press, pp265–301, 1987.
2. Bogo V. Behavioral radioprotection. In: Weiss JF, Simic MG, Eds. Perspectives in Radioprotection. New York: Pergamon Press, pp73–78, 1988.
3. Landauer MR, Davis HD, Dominitz JA, Weiss JF. Comparative behavioral toxicity of four sulfhydryl radioprotective compounds in mice: WR-2721, cysteamine, diethyldithiocarbamate, and n-acetylcysteine. In: Weiss JF, Simic MG, Eds. Perspectives in Radioprotection. New York: Pergamon Press, pp97–100, 1988.
4. Bogo V, Jacobs AJ, Weiss JF. Behavioral toxicity and efficacy of WR-2721 as a radioprotectant. Radiat Res **104**:182–190, 1985.
5. Bogo V, Franz CG, Jacobs AJ, Weiss JF, Young RW. Effects of ethiofos (WR-2721) and radiation on monkey visual discrimination performance. In: Weiss JF, Simic MG, Eds. Perspectives in Radioprotection. New York: Pergamon Press, pp93–95, 1988.
6. Glover D, Fox KR, Weiler C, Kligerman MM, Turrisi A, Glick JH. Clinical trials of WR-2721 prior to alkylating agent chemotherapy and radiotherapy. In: Weiss JF, Simic MG, Eds. Perspectives in Radioprotection. New York: Pergamon Press, pp3–7, 1988.
7. Glover D, Riley L, Carmichael K, Spar B, Glick J, Kligerman MM, Agus ZS, Slatopolsky E, Attie M, Goldfarb S. Hypocalcemia and inhibition of parathyroid hormone secretion after administration of WR-2721 (a radioprotective and chemoprotective agent). N Engl J Med **309**:1137–1141, 1987.
8. Kligerman MM, Glover DJ, Andrew TT, Norfleet AL, Yuhas JM, Coia LR, Simone C, Glick JH, Goodman RL. Toxicity of WR-2721 administered in single and multiple doses. Int J Radiat Oncol Biol Phys **10**:1773–1776, 1984.

9. Walker RI. Requirements of radioprotectors for military and emergency needs. In: Weiss JF, Simic MG, Eds. Perspectives in Radioprotection. New York: Pergamon Press, pp13–20, 1988.
10. Sweeney TR. A Survey of Compounds from the Antiradiation Drug Development Program of the U.S. Army Medical Research and Development Command. Washington, DC: Walter Reed Army Institute of Research, 1979.
11. Brown DQ, Grahm WJ, MacKenzie LJ, Pittock JW, Shaw LM. Can WR-3689 be improved upon? In Weiss JF, Simic MG, Eds. Perspectives in Radioprotection. New York: Pergamon Press, pp157–168, 1988.
12. Landauer MR, Davis HD, Kumar KS, Weiss JF. Behavioral toxicity of selected radioprotectors. Adv Space Res **12**:273–283, 1992.
13. Kumar KS, Weiss JF, McLean WA, Kendrick JM, Landaur MR. Radioprotection by combinations of WR-3689 and caffeine. Abstracts of Papers for the Thirty-Eighth Annual Meeting of the Radiation Research Society, p193, 1990.
14. Lloyd KG, Homyliewicz O. Catecholamines in regulation of motor function. In: Friedhoff AJ, Ed. Catecholamines and Behavior. New York: Plenum Press, Vol **1**: pp41–57, 1975.
15. Rall TW. Central nervous system stimulants (continued), the methylxanthines. In: Gilman AG, Goodman LS, Rall TW, Murad F, Eds. The Pharmacological Basis of Therapeutics (7th ed). New York: Macmillan Publishing, pp589–603, 1985.
16. Berkowitz BA, Tarver JH, Spector S. Release of norepinephrine in the central nervous system by theophylline and caffeine. Eur J Pharmacol **10**:64–71, 1970.
17. Corrodi H, Fuxe K, Jonsson G. Effects of caffeine on central monoamine neurons. J Pharm Pharmacol **24**:155–158, 1972.
18. Waldeck B. Ethanol and caffeine: A complex interaction with respect to locomotor activity and central catecholamines. Psychopharmacologia **36**:209–220, 1974.
19. Waldeck B. On the interaction between caffeine and barbiturates with respect to locomotor activity and brain catecholamines. Acta Pharmacol Toxicol **36**:172–180, 1975.
20. Schlosberg AJ, Fernstrom JD, Kopczynski MC, Cusack BM, Gillis MA. Acute effects of caffeine on neutral amino acids and brain monoamine levels in rats. Life Sci **29**:173–183, 1981.
21. Zielke HR, Zielke CL. Lack of sustained effect on catecholamines or indoles in mouse brain after long term subcutaneous administration of caffeine and theophylline. Life Sci **39**:565–572, 1986.
22. Govani S, Petkov VV, Montefusco O, Missale C, Battaini F, Spano PF, Trabucchi M. Differential effects of caffeine on dihydroxyphenylacetic acid concentrations in various rat brain dopaminergic structures. J Pharm Pharmacol **36**:458–460, 1983.
23. Minana MD, Grisolia S. Caffeine ingestion by rats increases noradrenaline turnover and results in self-biting. J Neurochem **47**:728–732, 1986.
24. Waldeck B. Some effects of caffeine and aminophylline on the turnover of catecholamines in the brain. J Pharm Pharmacol **23**:824–830, 1971.
25. Karaswa T, Furukawa K, Yoshida K, Shimizu M. Effect of theophylline on monoamine metabolism in the rat brain. Eur J Pharmacol **37**:97–104, 1976.
26. Galloway MP, Roth RH. Neuropharmacology of 3-isobutylmethylxanthine: Effects on central noradrenergic systems *in vivo*. J Pharmacol Exp Ther **227**:1–8, 1983.
27. Palazzolo DL, Quadri SK. Reduced variation in retention times of biogenic amines by temperature control in liquid chromatography with electrochemical detection. J Chromatogr **479**:216–219, 1989.
28. Palazzolo DL, Quadri SK. Optimal conditions for long term storage of biogenic amines for subsequent analysis by column chromatography with electrochemical detection. J Chromatogr **518**:258–263, 1990.
29. Rasey JS, Nelson NJ, Mahler P, Anderson K, Krohn KA, Menard T. Radioprotection of normal tissue against gamma rays and cyclotron neutrons with WR-2721: LD_{50} studies and ^{35}S-WR-2721 biodistribution. Radiat Res **97**:598–607, 1984.
30. Rasey JS, Krohn KA, Grunbaum Z, Spence AM, Menard TW, Wade RA. Synthesis, biodistribution, and autoradiography or radiolabeled S-2-(3-methylaminopropylamino)ethylphosphorothioic acid (WR-3689). Radiat Res **106**:366–379, 1986.
31. Dobric S, Milovanovic SR, Tanasijevic D. The effects of gamma-irradiation on the activity of the isolated perfused heart, isolated uterus, and on the amount of monoamines in the brain of the rats pretreated by WR-2721. In: Proceedings of the 30th Anniversary Symposium of Radiation Protection in the Boris Kidric Institute of Nuclear Sciences, Belgrade, pp244–249, 1989.
32. Deanovic ZBM, Isgum V, Geber J. Average evoked potentials and acetylcholine release in the somatosensory cortex of the cat treated with WR-2721. In: Proceedings of the 30th Anniversary Symposium of Radiation Protection in the Boris Kidric Institute of Nuclear Sciences, Belgrade, pp137–144, 1989.
33. Purdie JW, Inhaber ER, Schneider H, Labelle JL. Interaction of cultured mammalian cells with WR-2721 and its thiol, WR-1065: Implications for mechanisms of radioprotection. Int J Radiat Biol **43**:517–527, 1983.
34. Allalunis-Turner MJ, Walden TL, Sawich C. Induction of marrow hypoxia by radioprotective agents. Radiat Res **118**:581–586, 1989.
35. Gibson GE, Pulsinelli W, Blass JP, Duffy TE. Brain dysfunction in mild to moderate hypoxia. Am J Med **70**:1247–1254, 1981.
36. Gordon K, Statman D, Johnston MV, Robinson TE, Becker JB, Silverstein FS. Transient hypoxia alters striatal catecholamine metabolism in immature brain: An *in vivo* microdialysis study. J Neurochem **54**:605–611, 1990.
37. Pastuszko A, Wilson DF, Erecinska M. Neurotransmitter metabolism in rat brain synaptosomes: Effect of anoxia and pH. J Neurochem **38**:1657–1667, 1982.
38. Nagatsu T. Biochemistry of Catecholamines. Baltimore: University Park Press, pp71–73, 1973.
39. Terry LC, Craig R. Cysteamine effects on monoamines, dopamine-β-hydroxylase and the hypothalamic-pituitary axis. Neuroendocrinology **41**:467–475, 1985.
40. Palazzolo DL, McLean WA, Kumar KS. Effects of S-2(3-methylaminopropylamino)ethylphosphorothioic acid (WR-3689) on the content of catecholamines in the mouse adrenals. Proc Soc Neurosci (Abstracts) **18**:1376, 1992.
41. Vujnov S, Simovic M, Cvetkovic M, Savic J. Effects of gammaphos and labetol on adrenaline level in the adrenals, plasma, and urine of rats subjected to irradiation injury. Iugosl Physiol Pharmacol Acta **26**:411–418, 1990.
42. Weiss JF, Kumar KS. Antioxidant mechanisms in radiation injury and radioprotection. In: Chow CK, Ed. Cellular Antioxidant Defense Mechanisms. Boca Raton, FL: CRC Press, Vol **II**: pp163–189, 1988.
43. Cook LL, Bissette G, Dole K, Nemeroff CB. A critical evaluation of cysteamine as a tool to deplete somatostatin in the rat central nervous system. Endocrinology **124**:855–861, 1989.
44. Sagar SM, Millard WJ, Martin JB, Murchison SC. The mechanism of action of cysteamine in depleting prolactin immunoreactivity. Endocrinology **117**:591–600, 1985.
45. Wise PM. Changes in hypothalamic catecholamines associated with aging and reproductive functions. In: Ben-Jonathan N, Bahr JM, Weiner RI, Eds. Catecholamines as Hormone Regulators. New York: Raven Press, pp51–62, 1985.
46. Douglas WW, Poisner AM. Stimulus-secretion coupling in a neurosecretory organ: The role of calcium in the release of vasopressin from the neurohypophysis. J Physiol **172**:1–18, 1964.
47. Foreman MM, Porter JC. Prolactin augmentation of dopamine and norepinephrine release from superfused medial basal hypothalamic fragments. Endocrinology **108**:800–804, 1981.

48. Hartter DE, Ramirez VD. The effects of ions, metabolic inhibitors, and colchicine on luteinizing hormone-releasing hormone release from superfused rat hypothalami. Endocrinology **107**:375–382, 1980.
49. Porter JC, Aguila-Mansilla N, Ramin SM, Kozlowski GP, Kedzierski W. Tyrosine hydroxylase expression in hypothalamic cells: Analysis of the roles of adenosine 3′,5′-monophosphate- and Ca^{2+}/calmodulin-dependent protein kinases in the action of pituitary cytotropic factor. Endocrinology **129**:2477–2485, 1991.
50. Ebstein RP, Daly JW. Release of norepinephrine and dopamine from brain vesicular preparations: Effects of adenosine analogues. Cell Mol Neurobiol **2**:193–204, 1982.
51. Carpenter MB, Sutin J. Human Neuroanatomy (8th ed). Baltimore: Williams & Wilkins, 1983.
52. Gale CC. Neuroendocrine aspects of thermoregulation. Annu Rev Physiol **35**:391–430, 1973.
53. Carpenter DO. Neural mechanisms of emesis. Can J Physiol Pharmacol **68**:230–236, 1990.
54. Kandasamy SB, Kumar KS, Hunt WA, Weiss JF. Opposite effects of WR-2721 and WR-1065 on radiation-induced hypothermia: Possible correlation with oxygen uptake. Radiat Res **114**:240–247, 1988.
55. Polla BS, Donati Y, Kondo M, Tochon-Danguy HJ, Bonjour JP. Protection from cellular oxidative injury and calcium intrusion by N-(2-mercaptoethyl)-1,3-propanediamine, WR-1065. Biochem Pharmacol **40**:1469–1475, 1990.
56. Weaver ME, Morrissey J, McConkey C, Goldfarb S, Slatopolsky E, Martin K. WR-2721 inhibits parathyroid adenylate cyclase. Am J Physiol **252**:E197–E201, 1987.
57. Nakamoto T, Joseph F Jr, Yazdani M, Hartman AD. Effects of different levels of caffeine supplemented to the maternal diet on the brains of newborn rats and their dams. Toxicol Lett **44**:167–185, 1988.
58. Nakamoto T, Joseph F Jr. Interaction between caffeine and zinc on brain in newborn rats. Biol Neonate **60**:118–126, 1991.